niche demonstrated exacerbated loss of ISC in IL-22 KO mice during GVHD.

In summary, IL-22 was produced post-BMT by host ILC that were eliminated during GVHD, and IL-22 deficiency increased GVHD morbidity and mortality. While IL-22 deficiency did not significantly alter the donor immune response, it did lead to increased GVHD pathology, loss of epithelial integrity, and loss of ISC. IL-22 is thus critical for protection of host epithelium during GVHD. This may be exploited in the future to reduce clinical GVHD without limiting the curative potential of the transplant.

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SYSTEMATIC EVALUATION OF GRAFT-VERSUS-LEUKEMIA IMMUNITY OF AML-REACTIVE T CELL PRODUCTS USING HUMANIZED NOD/SCID/IL2R γC^{NULL} MICE

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Introduction: We have recently established an immunodeficient NOD/SCID/IL2Rγc^{null} (NSG) mouse model that allows the reliable engraftment of primary human acute myeloid leukemia (AML) blasts, particularly those with FLT3-ITD mutations. We now use these mice as a platform to evaluate the graft-versus-leukemia (GvL) effect of human leukemia-reactive T cells in vivo. To systematically optimize essential parameters for survival and effector function of human T cells in NSG mice, large numbers of AML-reactive cytotoxic T lymphocytes (CTL) were generated in vitro by stimulation of healthy donor $CD8^+$ T cells with FLT3-ITD^+ AML blasts in either single HLA-class I-mismatched or HLA-matched situations.

Methods: 5x10e5 AML blasts were injected into 6-8 week-old irradiated (150cGy) NSG mice to achieve 1-5% AML engraftment in bone marrow (BM) within 18 days (d) resembling minimal residual disease. Subsequently, 5x10e6 alloreactive $CD8^+$ CTL expanded over 14, 21, 28, and 56 d in vitro were transfused into mice to investigate differences in homing, survival, and GvL reactivity in vivo. Controls included AML-engrafted mice without CTL as well as mice receiving CTL of irrelevant antiviral specificity. All mice received human interleukin (IL)2, IL7-Fc and IL15 at time of T cell injection. AML-reactivity was analyzed in kinetic studies 2 h, 24 h, 48 h and 7 d after CTL transfer.

Results: We observed complete eradication of patient-derived FLT3-ITD^+ AML blasts in BM, spleen and peripheral blood of mice one week after transfer of single locus HLA-B mismatched CTL that had been cultured for 14, 21, or 28 d, respectively. In contrast, control mice showed 25-61% (median 35%) leukemia infiltration in BM. Kinetic analysis demonstrated almost complete AML remission as early as 48 h after T cell transfer. Ex vivo analysis of CTL re-isolated from murine spleens 24 and 48 h after injection showed persistent reactivity to AML blasts, but not to NSG-derived murine dendritic cells. Interestingly, CTL expanded over 56 d in vitro appeared less capable to eradicate AML in vivo. Results were reproducible in 2 different donor-patient pairs and, moreover, are in line with ongiong studies in HLA-matched systems.

Conclusion: We show herein that NSG mice engrafted with primary human AML blasts can be successfully treated with human alloreactive CTL. The model will be further optimized to serve as a general platform for testing the GvL effect of T cell grafts before adoptive transfer into humans.

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PRELIMINARY RESULTS OF A PHASE II TRIAL OF MONTELUKAST FOR THE TREATMENT OF BRONCHIOLITIS OBLITERANS SYNDROME AFTER HSCT

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Bronchiolitis obliterans syndrome (BOS) after allogeneic HSCT is a deadly manifestation of cGVHD. Current treatments are inferior and yield transient responses with published overall survival of 44% at 2 years. Although the pathogenesis of BOS after HSCT is unknown, a similar disease, BOS after lung transplant is associated with elevated leukotriene levels. We present preliminary results from a prospective, open label, phase II trial testing the efficacy of montelukast, a leukotriene inhibitor, for the treatment of BOS after HSCT. BOS diagnostic criteria included: FEV1<75%, FEV1/VC < 0.7 or air trapping on CT and RV>120% or RV/TLC>120% in the absence of infection and presence of another cGVHD manifestation. Subjects had stable or declining FEV1 on stable or decreasing immunosuppression. Twenty patients have been enrolled. One withdrew prior to treatment and one withdrew after study medication initiation; 16/20 patients have reached the primary endpoint (6 months) on study medication (10 mg qhs). Study participants age ranged from 15-64 years, 12/20 female, with baseline FEV1 from 24 to 73% predicted. All patients met criteria for response on the clinical trial with less than 15% decline in FEV1 % predicted at the primary endpoint. FEV1 increased 5-13% predicted in 5 participants, remained stable in 6 (change <5%), and declined 5-13% in 5. Comparison of patient pre-study FEV1 decline to on-study FEV1 values was generated using the slope of FEV1 volume vs. days post-transplant. The difference in pre- and primary endpoint slope revealed: 14/16 improvement and 2/16 decline. Six minute walk test demonstrated that 4/16 patients had significant increases in walk distance that exceeded the minimally important difference, 2 of which had declining FEV1%. 2/20 had a significant decline in walk test accompanied by a decline in FEV1% predicted. Of 10 patients eligible for the 2 year endpoint, 7/10 are alive with 2 patients with durable FEV1 improvements from baseline (FEV1+ 6%, +14%), 2 stable (FEV1 0%, +1%), 3 with decline from baseline (FEV1 -4%, -4%, -8%). Montelukast was well-tolerated with only one grade II probable attributable adverse event (insomnia) during the six-month collection period. These findings suggest that montelukast is a promising therapy for BOS after allogeneic HSCT.

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ALLOANTIGEN PRESENTATION BY RECIPIENT NON-PROFESSIONAL ANTIGEN PRESENTING CELLS INDUCES LETHAL ACUTE GVHD

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Aim: The immunological parameters governing the presentation pathways for allogeneic peptides to induce graft-versus-host disease (GVHD) are unclear. This is critical for the development of clinical therapies based on antigen presentation to control deleterious alloreactive responses.

Method and Result: We developed a GVHD model following a bone marrow transplant (BMT) system whereby presentation of a processed recipient peptide within MHC class II can be spatially and temporally quantified. The presentation of peptide within MHC class II by recipient antigen presenting cell (APC) resulted in GVHD mortality within ten days. While donor APC could induce lethal acute GVHD, recipient APC were 100-1000 times more potent. Antigen presentation by recipient APC resulted in accumulation of antigen-specific T cells within

the gastrointestinal tract and severe histopathology. The specific deletion of recipient dendritic cells surprisingly enhanced the expansion of donor alloantigen-specific T cells and accelerated GVHD mortality due to a failure of activation-induced donor T cell death. Consistent with this, the use of bone marrow-chimeric recipients demonstrated that professional, hematopoietic-derived recipient APC in isolation were limited in their capacity to induce GVHD. In contrast, non-hematopoietic recipient APC in isolation induced universal GVHD mortality with high levels of alloreactive donor T cell expansion and inflammatory cytokine generation. Confocal imaging demonstrated that MHC class II is highly expressed in recipient non-hematopoietic tissue within the dermis and intestinal villi. Donor T cell activation (CD69) and memory differentiation ($CD62L^{lo}/CD44^{hi}$) occurred in irradiated recipients in an antigen-independent fashion and resulted in the acquisition of a memory cell phenotype and Th1 differentiation in lymph nodes. Within 3 days after BMT, these donor T cells began entering the GI tract and interacted with MHC class II^{+} non-hematopoietic cells leading to lethal acute GVHD.
Conclusion: These data challenge current paradigms, demonstrating that lethal acute GVHD can be induced by alloantigen presented solely within MHC class II by non-hematopoietic recipient APC.

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MEASUREMENT OF ORAL CHRONIC GRAFT-VERSUS-HOST DISEASE

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Oral chronic graft-versus-host disease (cGVHD) is a serious complication of allogeneic stem cell transplantation, and may be the primary site of disease activity. Scales and instruments recently introduced to measure severity and response to therapy have not been prospectively validated. The objective of this study was to describe the characteristics of oral cGVHD and determine the measures most sensitive to change.
Methods: Patients enrolled in the cGVHD Consortium with oral involvement were included. Clinicians scored oral cGVHD according to the 2005 NIH criteria for severity scoring (0-3) and response (erythema, lichenoid changes, ulcers, mucoceles), and patients completed measures. Other evaluated measures included the esophageal response measure, Hopkins mouth score, and weight. Clinicians and patients also rated change on an 8-point scale, categorized as improved (1-3), stable (4-6), or worsened (7-8).
Results: Of 458 participants with cGVHD, 72% (n = 331) had oral cGVHD involvement at enrollment and were followed for a median of 13.6 months (2.0-38.5). Lichenoid change was the most common objective finding (n = 293; 89%), and 25% of patients had only lichenoid involvement. Oral cGVHD was not associated with global quality of life as measured by the FACT-BMT or SF-36. At visits where change could be assessed (n = 501, 52% of follow-up visits), 51% of clinicians and 56% patients reported improvement, with worsening reported in 4-5% for both groups; agreement between clinician and patient perceived change was fair (weighted kappa = 0.41), but only 1% of visits had highly discordant changes (improve vs. worse). Multivariable regression modeling suggested that the serial measurement changes most predictive of perceived change by clinicians and patients were the erythema and lichenoid scores, NIH severity score, clinician assessed pain score and patient assessed Lee oral symptom score. Serial change in erythema and lichenoid features showed synergy, with more combined impact than each feature alone. Perceived changes in oral cGVHD were not associated with change in ulcers, mucoceles, esophageal scores or patient weight.
Conclusions: Oral involvement in cGVHD is common and associated with a wide range of signs and symptoms that generally improve with time. Measurement of oral erythema and lichenoid changes, pain and patient symptoms using 6 questions may adequately capture the activity of oral cGVHD in clinical trials.

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REGULATION OF INTESTINAL INFLAMMATION BY INTESTINAL MICROBIOTA FOLLOWING ALLOGENEIC BONE MARROW TRANSPLANTATION

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Following allogeneic bone marrow transplantation (allo BMT), patients are at high risk for developing intestinal inflammation secondary to graft-versus-host disease (GVHD). While the impact of the microbiota on GVHD is known to be significant, no consensus exists between BMT centers regarding the optimal approach to target the flora.

We first examined in mouse models the effects of GVHD on gut flora. While BMT alone produced surprisingly few changes in the flora of mice, with GVHD we observed loss of overall diversity, and in particular expansion of Lactobacillales and loss of Clostridiales. We studied the effects of eliminating Lactobacillales from the flora of mice prior to BMT using antibiotics and observed aggravation of GVHD, while re-introducing the predominant species of Lactobacillus mediated significant protection resulting in improved survival. These results from murine models suggest that GVHD produces unique changes in the flora, and that changes induced by antibiotics can aggravate GVHD.

We then characterized gut flora of eight patients undergoing allo BMT during onset of intestinal inflammation due to GVHD, compared to ten patients without GVHD. We again found patterns of loss of diversity, expansion of Lactobacillales, and loss of Clostridiales, mirroring our findings in mice. We also identified increased microbial chaos early following allo BMT as a potential risk factor for subsequent GVHD. Together, these data increase our appreciation for reciprocal regulation of inflammation and flora in the intestine, and suggest that flora manipulation may improve outcomes for allo BMT recipients.

Table. Summary of GVHD-induced changes in the microbiota

	Mice		Humans	
	No GVHD	GVHD	No GVHD	GVHD
Flora diversity	no change	decreased	no change	decreased
Lactobacillales	no change	increased	no change	increased
Clostridiales	no change	decreased	no change	decreased

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IDENTIFICATION OF A NEW HY MINOR IN THE UTY GENE USING REVERSE IMMUNOLOGY

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Introduction: Minor histocompatibility antigens located on the Y-chromosome (HY minors) are known to play a pivotal role in allogeneic hematopoietic cell transplantation (HCT) with female donor and male recipient. Only a few HY minors are known. We identified a new HY minor using reverse immunology where candidate minors first were predicted using bioinformatics and afterwards confirmed with standard immune laboratory techniques.
Methods: Patient/donor pairs with female donors and male patients were high resolution HLA typed. Candidate HY minor epitopes located in genes only expressed on the Y chromosome were found using the HLA-pan restrictor (http://www.cbs.dtu.dk/services/HLArestrictor). Post nonmyeloablative conditioning HCT PBMCs from the patients were thawed, stimulated with these peptides and tested for cytokine produktion (TNF-α and IFN-γ) after restimulation using flow cytometri. Approximately 140 peptides were synthesized per patient/donor pair and the test for cytokine production was carried out using a matrix system. When positive cytokine responses were found, the optimal peptide and the HLA-restriction were determined by affinity assays and tetramerstaining. Cytotoxity was demonstrated by staining for CD107a.
Results: A strong cytokine response on an 11-mere peptide located in the UTY gene was found in a patient transplanted for follicular